Abstract

Chromatographic separations are often characterized by multiple detectors though which the sample flows serially. As the sample flows between detectors, it becomes progressively diluted due to mixing and diffusion. This phenomenon is traditionally called "band broadening" and often results in significant distortion of the calculated physical properties such as molar mass and size. This is particularly true for the case of monodisperse samples such as proteins. A new procedure is described whereby most types of band broadening may be corrected resulting in more accurate calculations of such physical properties. The conventional means for correcting band broadening effects is based upon mathematical procedures that attempt to narrow the broadened peak to its prebroadened form. Such procedures are notoriously unstable and often result in unphysical results such as ringing, negative concentrations, or negative scattered intensities. This disclosure describes a method to characterize the broadening present in a chromatographic system, and an algorithm whereby the narrow peaks of the upstream detector are artificially broadened so that they can be compared to the broadened peaks of the downstream detector. Although the technique results in some loss of resolution, its stability and generality allow it a broad range of application. Examples include correction of RI detector broadening following MALS detectors, correction of MALS broadening following UV detection, correction of viscometric broadening following both MALS and RI detection, and all permeations thereof.